ANTHECOLOGICAL RELATIONS BETWEEN REPUTEDLY ANEMOPHILOUS FLOWERS AND SYRPHID FLIES

I. THE POSSIBLE ROLE OF SYRPHID FLIES AS POLLINATORS OF *PLANTAGO*

by

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With two figures and three plates

Abstract

Regular visits by syrphid flies of the genera *Melanostoma* and *Platycheirus* to the inflorescences of the reputedly anemophilous *Plantago lanceolata* L. for the purpose of pollen consumption have often been reported. An effective pollen transfer could be established in the present study. Observations made in the field indicated that the flies feed on *Plantago laceolata* pollen in the early morning. Several aspects of their behaviour already suggest them to be effective pollen vectors. By means of Scanning Electron Microscope observations the presence of pollen grains of *P. lanceolata* on the body of the flies could be demonstrated. Experiments in which artificially dyed pollen of *P. lanceolata* was deposited on inflorescences in anthesis, while the stained pollen was subsequently recovered from the stigmas of other spikes, demonstrate that they are efficient pollinators of this plant. The amount of pollen transferred was estimated and, at least in certain habitats, is thought to be responsible for a considerable part of the geitonogamous and heterogamous pollinations. The technique employed may prove to be useful for the study of comparable cases in plants and animals.

I. Observations in the field and preliminary studies

The taxon Syrphidae (Diptera) almost exclusively consists of species which in the adult stage are typically anthophilous and feed chiefly on pollen or nectar. Differences in body size, and in the length of the proboscis, are considerable, so that a rather wide range of flowers can be visited. Among the Diptera, the Syrphidae are in general the most important pollinators, although their significance is not as great as that of the social hymenopterous group of the Apidae (Kugler, 1970; Faegri & Van der Pijl, 1971).

As may be expected, syrphid flies normally visit flowers or inflorescences of typically entomophilous plants, but observations in the field have shown that some reputedly anemophilous plants are visited regularly by various species of Syrphidae for the purpose of pollen consumption. However, the published records provide relatively few reliable data. The most important ones, to be mentioned presently, refer mostly to visits by representatives of the closely related genera *Melanostoma* and *Platycheirus* of the subfamily Syrphinae.

One of the earliest reports is by Müller (1873), who noted that in the surroundings of Lippstadt (W. Germany) insects often gather or consume pollen on the spikes of *Plantago lanceolata*. Beside Hymenoptera, he reported several

Syrphidae among which *Melanostoma mellinum* (Linnaeus) is specially mentioned as a frequent pollen-eating visitor. Müller pointed out that this fly even shows a preference for wind-pollinated flowers: he found this insects as a regular pollen consumer on *Plantago media* L., *Artemisia dracunculus* L., *Scirpus lacustris* L., and several grasses such as *Anthoxanthum odoratum* L., *Poa annua* L., and *Festuca pratensis* Huds. It was also found on a whole range of entomophilous plants where, apart from pollen, it sometimes also ingested nectar. Müller believed that visits to anemophilous plants by this type of insects might result in cross-pollination. Representatives of the closely related *Platycheirus* were not reported as visitors of wind-pollinated taxa.

Ludwig (1881, 1884) noticed a very large number (several thousands) of syrphids of the genera *Melanostoma, Platycheirus* and *Melithreptus (Sphaerophoria)* on the panicles of *Molinia coerulea* (L.) Moench in Saxony (E. Germany). An appreciable number were caught by the proboscis between the paleae or the anthers and in that case they usually appeared to be infected by a parasitic fungus (*Entomophtora* spec.), while living flies were often found to be present also. Ludwig suggested that the purplish-mauve colour of the anthers acts as a strong attractant for the visitors. He observed the same flies on *Phleum pratense* L., *Helictotrichon pubescens* (Huds.) Pilger, *Dactylis glomerata* L., and *Plantago lanceolata.*

The extensive monograph of Knuth (1898—1905) records a number of cases of *Melanostoma mellinum* visiting various anemophiles. Apart from citing earlier authors he also reported personal observations made near Kiel and in the North Frisian islands (W. Germany), where he frequently noted several specimens of this fly on inflorescences of *Alopecurus pratensis* L., *Phleum pratense,* and *Anthoxanthum odoratum.* He also mentioned visits of this species to *Plantago arenaria* W. & K. (in a botanical garden) and to *Sanguisorba minor* Scop. Knuth did not attribute any significant role to these visits in effective pollen transfer. In addition he mentioned an appreciable number of visits of *Melanostoma mellinum* (and other species of the genus) and of *Platycheirus* species to entomophilous flowers.

Drabble & Drabble (1927) reported that at one time (in Great Britain) they saw inflorescences of *Phleum pratense* literally covered with *Melanostoma mellinum* and *Hylemia variata* (Fallén) (Diptera, Anthomyidae), which were busy gathering pollen. They believed that these insects are undoubtedly effective pollinators. *Dactylis glomerata* was also visited for foraging purposes but its inflorescences do not seem to be as attractive as those of other grasses.

Porsch (1956) gave an extensive survey of Coleoptera and Diptera recorded as pollen consumers on anemophilous plant species, but his lists are chiefly compiled from earlier publications and do hardly contribute any new data on syrphids.

For our present study the publication by Van der Goot & Grabandt (1970) proved to be important. They studied the pollen content of the digestive tract of several anthophilous species of Syrphidae belonging to the genera *Melanostoma, Platycheirus,* and *Pyrophaena.* The senior author had repeatedly (in the Netherlands) observed these flies visiting the inflorescences of Gramineae and of *Plantago lanceolata.* In order to assess their possible preference for anemophilous plants the ingested pollen was studied. It appeared that the diet of several species, viz., *Platycheirus clypeatus* (Meigen), *P. fulviventris* Macquart, *P. scambus* (Staeger) and *P. angustatus* Zetterstedt, consisted almost exclusively, and that of others, such as *Melanostoma mellinum* (L.) and *M. scalare* (Fabricius), partly, of pollen of the above-mentioned anemophiles. (It may be mentioned in passing that Grabandt has found pollen of *Plantago* and of grasses in related syrphids collected in Canada; pers. comm.). In all other species of syrphid flies examined the diet normally consists of pollen of entomophilous plants, anemophilous pollen apparently only being taken occasionally.

All observations cited and pollen analyses clearly indicate a certain degree of consistency in the visits of the *Melanostoma-Platycheirus* group to several anemophiles, more particularly to *Plantago lanceolata* and a number of grasses.

The question arises whether these flies can indeed act as effective pollinators for these reputedly anemophilous plants and if so, what the effectiveness of pollen transfer by insects is in comparison to the anemophilous pollination. As mentioned above, some authors suggest that pollen transfer by insects may be of some importance, but this conclusion is solely based on recorded observations of visits. In our opinion such listings of visitors, although indispensable, are only indicative of a possible pollen transfer, and only an experimental analysis can lead to more definite conclusions. Such an approach has hitherto never been attempted, as far as can be ascertained.

The studies reported in the present paper were primarily intended to demonstrate the possible incidence of an effective pollen transfer by syrphid flies from one inflorescence to another, both through field studies and experimentally. Three methods were employed:

(1) Field studies, mainly centred around the patterns of behaviour of the syrphids, as far as important in connection with a possible pollen transfer;

(2) the use of the SEM technique to study the precise localisation of pollen grains attached to the flies after a visit;

(3) experiments with stained (marked) pollen to demonstrate actual pollen transfer in the field (chapter II).

For practical reasons it was deemed necessary, for the time being, to limit our investigations mainly to *Plantago lanceolata*. The results obtained by Van der Goot & Grabandt (1970) rendered the choice of the potential pollen vectors an easy one; the following and (locally) relatively common species seemed to be the most likely candidates: *Melanostoma mellinum, Platycheirus clypeatus, P. fulviventris,* and *P. scambus.*

Since it is often impossible to identify each species with certainty in the field, especially flying specimens and those paying a fleeting visit to an inflorescence being hard to recognize, the flies were, generally speaking, treated as a group. This admittedly is a somewhat weak element in our deductions, but we had no option. Exact identification is always possible when the specimen is caught and can be examined, but we refrained from this practice as much as possible. Not only is it a time-consuming procedure, but we also did not want to extract so many individuals from the ecosystem within the scope of our investigations. Identifications of killed specimens were reduced to an unavoidable minimum. Fortunately we have obtained the strong impression from our observations in the field that the behaviour of all species included in our study is very similar, and for the purpose of the present investigation identical. Whenever the terms "syrphids" or "flies" are used in the following text, unless stated otherwise, always representatives of the *Melanostoma-Platycheirus* group are meant.

The report covers investigations carried out in 1973 and 1974; the field work was carried out from about the beginning of May till the end of September. Low-lying sites near water offered good opportunities. The main areas for study selected were the Naardermeer and (less intensively) the Ankeveen broads. Incidental observations were also made elsewhere in the province of Noord-Holland. The SEM observations were made at the Laboratorium voor Electronenmicroscopie of the University of Amsterdam.

Observations in the field

Observations in the field indicate that syrphids habitually visit inflorescences of *Plantago lanceolata*. Spikes in the male stage of anthesis (*P. lanceolata* is protogynous) are predominantly visited in the early hours of the morning after sunrise. There is a marked correlation between the beginning of the (male) anthesis and the activity of the flies. Both processes seem to be temperature-dependant and commence in the temperature range between 10°—13°C, but may to some extent also be incited by other factors. As soon as in a population of *P. lanceolata* the male anthesis has begun, i.e., when the first stamens have emerged, a few syrphids begin to arrive and descend on the inflorescences to start feeding on pollen. Normally these events take place between 5.00 and 7.00 hrs, but on relatively cool mornings, or when bad weather conditions prevail, the period may shift to a later time in the morning.

In the beginning the male phase of anthesis develops slowly and the number of visitors remains limited. When the temperature rises and the weather is not too unfavourable, the anthesis rapidly becomes more general and the frequency of the visits also increases appreciably in a short time span. Every day there is a relatively short period of an optimum of visits which seldom exceeds a couple of hours and falls between about 6.00 and 11.00 hrs, but the frequency usually diminishes already before 10.00 hrs and only rarely stays high after 11.00. After the decrease the visits diminish in number, and soon become more or less incidental to cease completely at about 11.00 hrs; the flies become less active after feeding and descend to various parts of plants to rest, but especially in the beginning they often perform cleansing and preening movements with their legs.

The sequential phenomena just described are probably connected with the following circumstances:

(i) More or less constant and at least fairly favourable weather conditions during the early morning induce a certain regularity in both the course of anthesis and the frequency of the visits until the optimum is attained;

(ii) Dissipation of the pollen from the anthers principally by air currents (wind power!) results in the exhaustion of the pollen in the anthers which reduces their importance as a source of food;

(iii) The flies become satiated after having consumed a large quantity of pollen and start their resting phase.

Unfavourable weather in the morning, but sometimes during the previous evening or night, may bring about appreciable deviations from the regular pattern of anthesis and the visiting rhythm. Our observations concerning the male phase of anthesis agree almost completely with the results of Hyde & Williams (1946) obtained at Cardiff (Wales).

Continuous observation of a group of flowering plantains during which all visits were recorded revealed that some of the spikes in anthesis are markedly more frequently visited than most other ones and that a sometimes appreciable number of spikes is not visited at all. The cause of this selective preference is obscure. Quite regularly two or three flies are present on the same spike, but larger numbers are usually not encountered: eight specimens were seen on a single spike on only one occasion.

The pattern of behaviour of the flies suggests that some activities may be of importance for a possible transfer of pollen. This requires some presuppositions, viz.,

(a) that pollen grains indeed become attached to the body of the fly, and

(b) that the pollen can subsequently be transferred to the stigmatic area of a different flower.

Some characteristics of behaviour established on the basis of numerous observations are the following.

It appears that as a rule the syrphids fly in the direction opposite to the prevailing movement of the air immediately above the stand of vegetation. This mode of flight was used to advantage in experiments to be described in the second chapter.

The approach flight towards a spike in anthesis is not necessarily followed by a landing on the same spike; quite frequently the insect hovers in the air in front of it for a short while to proceed to a different inflorescence where the movement may be repeated before the fly decides to descend on a third spike, etc. The final landing is preceded by a series of brief thrusting movements aimed at the inflorescence. The fly usually alights directly upon the anther-bearing zones, but not rarely on the portion of the spike above the stamens in which only the female phase of anthesis has proceeded (i.e., on the stigma-bearing zone); in the latter case the fly walks downwards towards the stamen-bearing part of the inflorescence.

When manipulating the anthers to feed on the pollen, the fly is usually attached to some place on the spike by means of its second and third pairs of legs and clutches an anther with its front legs. It subsequently places its proboscis in the dehiscence fissure of a theca and works it over along its full length. As soon as the theca seems to become empty the anther is given a half turn by the front legs so that the opposite slit comes within reach of the tongue. After having thus systematically emptied an anther the fly grabs another one but may also move towards the stigma-bearing zone of the spike or take off. The syrphids seem to prefer freshly dehisced anthers, but somewhat older ones are also used for foraging, especially later in the morning. Pollen consumption is repeatedly interrupted by preening (preening and cleaning of the body also take place when the insects are resting after feeding as we have seen).

The duration of a single visit to a flowering spike varies considerably. From over 900 observations it could be deduced that visits lasting from less than 30 seconds to 2 minutes constitute about 50% of all vistis, and that 87% of the visits do not last longer than 15 minutes. Visits of longer duration were recorded now and then; two exceptional cases of visits, lasting 56 and 58 minutes, respectively, were noted.

During the working over of the anthers the flies may take up different positions. This may be of importance in connection with the possible contamination of the insect body with pollen and in some instances also with the possible deposition of pollen grains adhering to the body on pollen-receptive stigmas. The most characteristic postures are the following:

(i) the insect sits inside the stamen zone; contamination of different portions of the body with pollen is possible (Pl. 1 Fig. 1);

(ii) the fly takes up a practically vertical position distally of the stamen zone with the head facing the anthers downwards; pollen may adhere to the frontal part of the body, and the second and third pairs of legs may come into contact with receptive stigmas and thus transfer attached pollen grains (Pl. 1 Fig. 2);

(iii) the insect is seated on the already wilted portion of the inflorescence below the stamen-bearing zone in a vertical position with the head in the upward direction; pollen may adhere to the frontal parts of the body but also to its under surface (Pl. 1 Fig. 3);

(iv) the fly clings in a nearly horizontal position to one or more stamens in the lower part of the whorl of stamens; contamination of particularly the lower surface of the body with pollen is possible (Pl. 1 Fig. 4);

(v) in the case of spikes in which the zone of male anthesis has almost shifted to the top: the insect sits on the tip of the inflorescence in a more or less horizontal position; pollen grains will become attached to mainly the frontal parts of the body, and deposition can take place as long as receptive stigmas are present (Pl. 1 Fig. 5).

More or less intermediate positions occur frequently, and during feeding the fly may shift its position.

The syrphids are repeatedly found on the stigma-bearing zone of the inflorescence, more particularly in the following situations:

(a) the fly alights on this part and after a shorter or longer sojourn proceeds to the stamen-bearing zone; sometimes it stays for a while, usually cleaning and preening its body; (b) after feeding on the pollen the insect walks from the stamen-bearing zone towards the stigma-bearing part and usually stays there for some time preening its body; it sometimes returns to the male zone to resume pollen consumption, but may also fly away (Pl. 1 Fig. 6).

If the female phase of anthesis has not terminated, the legs or the ventral side of the body may get in touch with receptive stigmatic surfaces, which may result in pollen transfer. In the first situation described above (a) the chance of cross-pollination is greater than in the second (because in the latter case the pollen is likely to originate from the same spike).

SEM studies of the captured flies

After the presence of pollen grains on the bodies of flies captured immediately after a visit to a *Plantago* inflorescence had been established by examination under a binocular dissecting microscope, a more detailed study was made by means of SEM microphotographs. For practical reasons only attention was paid to the ventral surface of the body (including the extremities). The study served a dual purpose: the pollen grains present could be identified more accurately, and the distribution of the pollen across the fly could be studied.

Methods

The flies were captured when they were resting on vegetative parts of plants and never when they were sitting on inflorescences so as to avoid possible contamination with the large quantities of pollen shaken out of the anthers when one is handling the killing jar. For each fly a separate, clean jar was used to avoid indirect contaminations as much as possible. After a captured fly had become sufficiently desiccated it was mounted with its dorsal side on an object support by

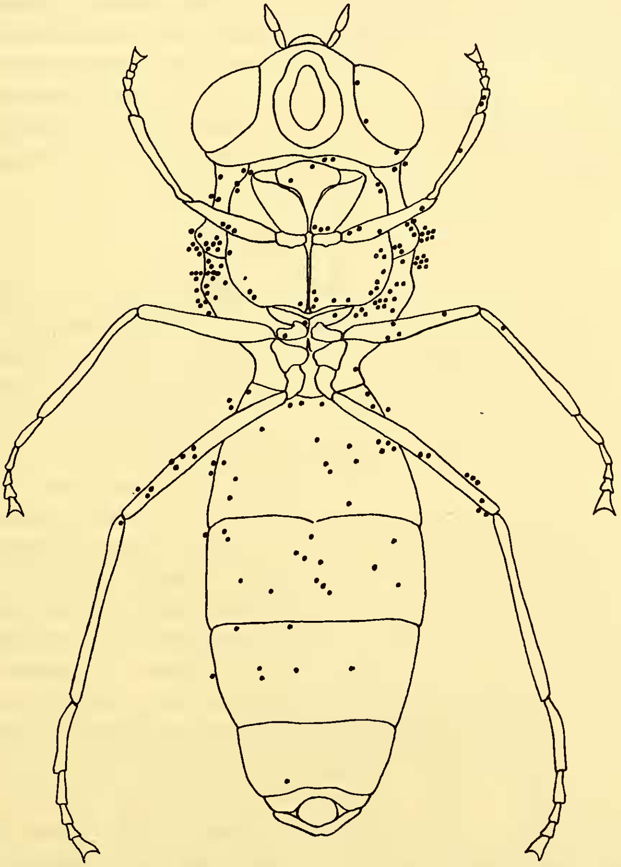

Fig. I. "Pollen map" of a syrphid fly (see text). The black dots represent pollen grains of *Plantago lanceolata* (dots situated outside the outline of the body represent pollen grains attached to setae protruding from the surface of the fly body) means of silver cement and coated with gold in an Edwards (model E 12 E) vacuum-coating apparatus. The coated specimens were systematically scanned for adhering pollen grains at suitable magnifications in a Cambridge Mark II Stereoscan (acceleration potential 10 KV). A schematic outline drawing of a fly was used to map the localisation and the species of the identified pollen grains; for each specimen a so-called pollen map (an example of which is shown in Fig. 1) was made in this way. Special and characteristic situations were photographically recorded.

Results

In all 32 syrphids were studied which belonged to all four species mentioned in the introduction. On all but one of these specimens the presence of pollen grains could be demonstrated. This pollen was almost exclusively of *Plantago lanceolata*; only in two cases a few gramineous grains were noted. The number of grains per fly varied a great deal: from a single one to a recorded maximum of 179 with a mean of 44. The localisation of the pollen on the ventral body surface was as follows: on the head only a few grains were observed, if any, and even around the mouth there were very few or none. The thorax carried the greatest number as a rule, especially in the pleural parts and on the hairs found in this part of the body. The abdomen bore fewer grains, mainly on the belly. Of the leg parts the femur carried the largest number of grains; between the three pairs of legs no important differences were found.

Discussion

The scanning technique demonstrated conclusively that pollen grains of *Plantago lanceolata* adhere to the bodies of the syrphid flies. The relatively few number of specimens studied do not permit an accurate assessment of the distribution of the pollen on the bodies of the flies, but it seems as if the head does not carry many grains. The grains are sometimes found on the glabrous cuticle, but more often adhere to the dermal setae and do not always touch the cuticle. The attachment is almost certainly attributable to a cementing substance, whose presence (in small amounts) in *Plantago* was shown by Pohl (1930) and Knoll (1930) almost simultaneously. On SEM microphotographs (compare Plate 2) the pollen grains, when lying close together, are frequently connected by a mass forming a conspicuous meniscus. Also between a grain sticking to a hair and the support such a substance is present. Pollen grains pretreated with ethanol before SEM examination do not show this phenomenon, so that it appears to be highly probable that the connecting matter is hardened pollen "*Kitt*" covered by a gold film. The almost complete absence of grass pollen on the flies is of special interest. Conceivably, its relatively great rarity has something to do with the incidental nature of visits to species of Gramineae for feeding purposes. It is also known that the surface of gramineous pollen grains is only very thinly covered with *Pollenkitt*, although the absolute quantity may vary appreciably from species to species (Pohl, 1930); this small amount of cementing substance may account for the poor sticking capacity of the grains (or for a short duration of their attachment during the flight of the insect). One must also bear in mind that the handling of the flies during the capture and their subsequent preparation may rub off a number of grains from the fly body, so that the SEM images do not necessarily present the situation immediately before the insects were caught: grass pollen may be rubbed off more easily than the plantain pollen.

Concluding remarks

Field observations of syrphid flies regularly feeding on *Plantago lanceolata* indicate that these insects may easily become contaminated with pollen. More casual visits to the inflorescences of grasses with easily accessible anthers may also result in the adherence of pollen to the body.

The efficiency of an insect as a pollinator is largely dependent on the constancy of its habits and its patterns of behaviour. If it frequently moves from one plant in anthesis to the inflorescence of a different individual of the same plant species the chance of transfer of specific pollen is appreciable. However, the efficacy of zoophilous pollination is also determined by the amount of pollen carried by the animal vector on its body, which amount differs from plant species to plant species owing to the varying quantities of pollen and to the presence or absence of other aids for the adherence of the grains to the insect body (such as a rough or spinulose ectexine). As far as can be deduced from the available data, grass pollen is less effectively transferred than plantain pollen, which may be interpreted as a greater reliance upon pollination by syrphid flies of *Plantago lanceolata* as compared to the grasses, at least in the area under investigation. This needs verification, and this relation should also be studied in other habitats.

The different amounts of plantain and grass pollen recorded on captured flies support the idea of a greater dependence of *Plantago lanceolata* on the flies in its pollination strategy. The repeated visits of syrphids to different individuals in stands of plantain render an efficacious pollen transfer highly probable. It also enabled the demonstration of the transfer of pollen from one plant to another to be dealt with in the next chapter.

II. EXPERIMENTS WITH STAINED POLLEN OF *Pantago lanceolata* L.

After the transfer of pollen of *Plantago lanceolata* by certain syrphid flies had been shown to be highly probable (see the first chapter), experiments were carried out to demonstrate the actual translocation of pollen grains from one plant to another by means of pollen marked by artificial staining. It has already conclusively been shown by means of SEM microphotographs that pollen grains adhere to various parts of the fly body, but although there were cogent indications of the role of the insects as effective pollen vectors, there remained some uncertainty whether a deposition by the flies of pollen grains on the receptive stigmatic surfaces of a flower in the female stage of anthesis actually takes place. By using artificially coloured grains partly replacing the normal pollen the identification of pollen grains deposited on other inflorescences was made possible, so that the displacement of this pollen could be traced. In addition, it was hoped that from such experiments certain ideas concerning the efficacy of a biotic pollen transfer under field conditions could be gleaned.

The marking of pollen by staining

Some preliminary tests had shown that pollen of *Plantago lanceolata* can readily be dyed by means of aqueous solutions of biological stains such as 0.5 % Methylene Blue and 0.5 % Neutral Red. Attempts to dye pollen grains still enclosed in the ripe anthers by spraying the inflorescences with such staining solutions were not very successful. Only a relatively small amount of pollen present near the slit of dehiscence appeared to have absorbed the dye, but the solution did not penetrate far enough. The anther wall may be more or less water-repellent. It was decided to use a technique in which pollen collected in the field was dyed in the laboratory as follows.

Pollen from spikes bearing numerous stamens in anthesis was harvested by beating the spikes over a metal tin. To obtain a quantity sufficient for subsequent processing at least 20-30 inflorescences must be shaken out. A small quantity of the collected pollen is poured on a disc of filter paper covering the bottom of a petri dish and spread out evenly by means of a fine paint brush. The dish is sprayed with the dye solution from a flower sprayer until the filter paper has assumed the colour of the solution. The (open) dish is left to dry in a suitable place at room temperature, which takes 2-3 hrs. To assess the condition of the grains the dish is placed under a dissecting microscope and the grains are touched with a fine preparation needle: if they are loose and can be easily shifted (or even jump away) the pollen is sufficiently desiccated. It is subsequently transferred to a glass tube with a brush and can be kept for some time until needed.

Pollen treated in this way was examined for some properties required for the purpose of the present investigation:

(i) Stained and normal (= untreated) pollen grains observed under a microscope at magnifications of 100—200 diam. appear to be always clearly distinguishable from one another;

(ii) When marked pollen was poured on to receptive stigmatic surfaces and the stigmas were subsequently examined under a dissecting microscope, such treated pollen grains appeared to adhere firmly to the stigmatic papillae and were clearly distinguishable in situ from untreated grains;

(iii) When living syrphid flies of the taxa studied (*Melanostoma*, etc., see the first chapter of this paper) were placed in a tube with dyed pollen, left in the tube for several minutes, and narcotised for examination under the microscope at a magnification of about 40 diam., they proved to carry a substantial amount of pollen on their bodies; the localisation of the pollen grains was very much the same as in the case of contamination with untreated pollen.

The treated grains apparently do not differ appreciably from undyed ones in some properties essential for their transfer by flies. To remove possible remaining doubts, the power of adherence of untreated and of dyed pollen was tested by locking up syrphids in a petri dish containing a small amount of a mixture of untreated and dyed grains in a known quantitative relation. After a brief sojourn the flies were narcotised and their bodies were examined for adhering pollen. From counts of the two kinds of grains for each fly (42 specimens) the relation between untreated/dyed pollen was estimated.

The result was as follows:
mean ratio untreated/dyed pollen in the mixture: 4.41 ± 0.12 [mean ratio untreated/dyed pollen on the fly bodies: 5.18 ± 0.54].

Experiments in the field: methods

For the experimental set-up the following reasoning was followed (see also the situation sketch, Fig. 2): When of two groups of flowering plants in a sufficiently dense stand of *Plantago lanceolata* one (A) is used as the donor group by applying dyed pollen to the spikes in the manner to be described below, and the other group (B) is left undisturbed, a syrphid fly visiting one or several donor spikes is likely to become covered with pollen including a number of treated grains. If it subsequently alights on untreated spikes there is a distinct possibility of dyed pollen becoming deposited on the stigmas of these spikes, which can be established by examining such spikes under a dissecting microscope (Pl. 3 Fig. B).

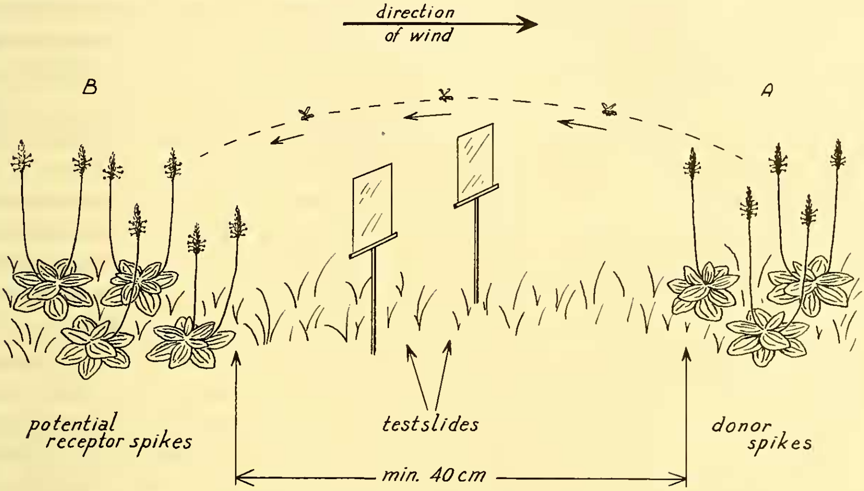

Fig. 2. Situation sketch of a field experiment (explanation in text). For the sake of clarity the rosette of leaves is drawn as if they are adpressed to the ground (as in *Plantago major* and *P. media*)

During the practical execution of the experiments the possible transfer of wind-borne pollen had to be taken into account. The relative position of the group of donor spikes was chosen in such a way that it always remained below the wind in respect of the untreated spikes present in the neighbourhood. A very favourable circumstance is that the syrphids tend to fly against the direction of the air current over the site, in the figure from group A towards group B. It goes without saying that this increases the efficacy and the reliability of the experiment.

In order to establish if nevertheless an undesired pollen transport of stained pollen took place, test slides were placed between the two groups which were coated with egg-white glycerol on the one side to catch air-borne pollen grains; these slides were placed at a height a little below the average height of the donor spikes, with the smeared side facing the latter. The distance between the groups of donor plants and receptor plants must not be too short (at least 40 cm) so as to avoid the risk of an undesired pollen transfer by direct contact between plants or by air turbulence. The area chosen for such experiments was always limited enough to be easily surveyable from one observation post and thus to permit the reliable recording of visits to receptor spikes by flies coming from donor spikes by following the flight of the latter.

After some trials a method was worked out to bring about a contamination of flies with dyed pollen which agrees to all intents and purposes with the mode of contamination with untreated pollen during a normal visit to an inflorescence. Dyed pollen is placed on a flowering spike as follows: A sufficient quantity of dyed pollen is put in a glass tube of about 10 cm long and 1.5—2 cm in diam. By shaking and tapping the tube whilst turning it about its longitudinal axis in a nearly horizontal position the pollen is evenly distributed over the inner wall. Such a tube is placed over a spike in male anthesis with a well-developed zone of stamens, the inflorescence during this and the subsequent operations being held in a horizontal position by bending its stalk carefully. By pushing the spike to and fro and, if necessary, by turning the tube a bit, some of the dyed pollen is rubbed off and sticks mainly to the anthers (but also to other parts of the inflorescence such as the stigmatic zone). The spikes must be dry; damp ones moisten the inside of the tube too much so that the pollen sticks too firmly to the tube and becomes useless. Such a treatment of the inflorescences does not deter flies from visiting them normally, even if the whorl of stamens has assumed a somewhat unnatural colour. The dyed pollen grains are as readily consumed as untreated ones; in a number of cases a marked bluish-green discolouration of the abdomen of a fly was observed after the animal had been feeding copiously on spikes treated with stained pollen. It may, therefore be taken for granted that the deposition of marked pollen grains on a spike does not affect the normal behavioural pattern of the flies in the least. The added pollen is also dispersed in a normal way by air currents as could be deduced from the gradual disappearance of the greyish discolouration, after some time, of a spike treated with pollen dyed blue, even if no insect visits took place. Examination of flies captured after they had visited a donor spike revealed the presence of coloured pollen grains on the fly body (Pl. 3 Fig. A).

Experiments in the field: results

Altogether 22 experiments were carried out, the duration of each single experiment being 60 to 150 minutes. The number of (treated) donor spikes was 3-6 and the receptor spikes usually numbered 3—4. Of about 90 spikes which were gathered in the field and might carry stained pollen grains ultimately only 67 could be used for the records, because some mishaps occurred which rendered them unusable (some became soaked with water, etc.).

The following qualifications need some explanation.

Fresh stigmas: stigmas with a shiny, somewhat vitreously white appearance; the distal parts may be suffused with brown.

Old stigmas: stigmas which have mostly or completely turned brown and have often become desiccated.

The duration of the receptive phase of the stigmas is not exactly known, but nevertheless the distinction made above, based on a different appearance, corresponds most probably with the receptive phase and the phase of post-anthesis of the stigmas.

After the experiments were concluded, a publication by Zeisler (1938) came to our notice in which a simple method is described to assess the degree of 'maturation' (receptiveness) of the stigmas by a chemical reaction (with H_2O_2). If this test is applicable to *Plantago* stigmas, some relevant additional information may thus be acquired.

Whole spike: includes bracts, perianth members, stigmas, and a zone of stamens.

$$\text{Stigma ratio (s.r.): } \frac{\text{number of stigmas with stained pollen grains}}{\text{total number of stigmas}}$$

Control with test slides: The pollen counts were made of a rectangular area of 10 mm by 20 mm in the middle of the slide at a magnification of 100—200 diam. Not in a single instance stained pollen was recorded, but a few undyed grains were usually present; the latter may have been deposited by air turbulence or convection currents, or as fall out of the local pollen rain from somewhat higher levels. In a number of cases test slides were also placed in a downwind position and these almost invariably had caught large quantities of both stained and unstained pollen.

Table 1. Presence or absence of dyed pollen on spikes of *Plantago lanceolata* L.

	Marked pollen present in	Marked pollen absent in
On fresh stigmas	43 spikes	24 spikes
On fresh and/or old stigmas	48 spikes	19 spikes
On whole spikes	50 spikes	17 spikes

The (67) receptor spikes were examined for the presence of marked pollen grains and the results are as follows (Table 1).

Table 2. Distribution of dyed grains over spikes of *Plantago lanceolata* L.

Number of dyed grains	Number present		
	On fresh stigmas	On old stigmas	On whole spike
(0)	(24)	(19)	(17)
1-10	25	15	11
11-20	8	14	11
21-50	7	14	18
51-100	2	2	5
more than 100	1	3	5
	—	—	—
	43	48	50

The following survey shows the number of individual grains in the cases in which dyed pollen had been transferred (Table 2). In the accompanying tabel (Tabel 3) the stigma ratio of 41 spikes with transferred dyed pollen grains, and the number of grains on the stigmas is shown. Of two of the 43 spikes with stained pollen grains the number of fresh stigmas was inadvertently not counted.

Table 3. Stigma ratio (s.r.) and number of grains on 41 spikes with transferred dyed pollen grains

s.r.		number of grains	s.r.		number of grains	s.r.		number of grains
3/9	-	8	10/10	-	136	1/1	-	3
1/10	-	4	2/2	-	19	6/10	-	36
6/7	-	63	7/9	-	47	4/11	-	20
1/12	-	3	3/6	-	19	13/36	-	32
12/17	-	98	4/8	-	26	2/6	-	2
8/10	-	47	5/13	-	12	6/9	-	26
1/29	-	2	1/17	-	2	4/11	-	4
2/4	-	9	1/23	-	4	2/26	-	9
4/8	-	31	1/21	-	12	1/8	-	1
2/11	-	7	2/4	-	3	1/22	-	1
5/17	-	10	4/14	-	14	3/3	-	19
2/3	-	7	1/11	-	2	3/4	-	6
2/7	-	5	2/14	-	5	3/9	-	10
4/7	-	9	3/21	-	13			

Discussion

The examination of the test slides indicates that an abiotic transportation of pollen against the direction of the wind is negligible. The conclusion that can be drawn from the results of the first survey (Table 1) is that syrphid flies are instrumental in the transfer of pollen from one spike to another and can do this repeatedly. The absence of marked pollen grains on potential receptors can have several reasons, the most important being:

(a) that the spikes were not visited by flies, or

(b) that the spikes were visited, but by flies that did not carry stained pollen grains.

One can only speak of an effective pollination if pollen is deposited on fresh (and presumably receptive) stigmas. This was the case in 20 experiments and in 64% of the total number of spikes examined, so that the general conclusion remains valid. One of the surveys (Table 2) gives a good idea of the quantities of marked pollen grains transferred by the animal pollen vector, but the results do not require a special discussion.

From the viewpoint of efficacy of pollen transfer the number of stigmas on which dyed pollen had been deposited is important. Table 3 shows that there are appreciable differences in this respect, both in stigma ratio and in the number of stained grains. This is to be expected if one considers that a number of mutually independent factors is involved in the pollen transfer to the stigmas, viz.,

(a) the total number of visits to a spike,

(b) the duration of these visits,
(c) the mode of landing, behaviour and/or displacement of the pollen vector,
(d) the amount and the location of the pollen on the body of the visitor, and
(e) the number and the length of the stigmas on the particular spike.

The first two factors could be quantified by the registration of the visits, but there was no correlation with the quantity of deposited marked pollen. Matters were complicated by the uncertainty in many cases whether a visitor was indeed a carrier of dyed pollen. The dyed grains were especially deposited on the tips of the stigmas as may be expected because these protruding parts are the most likely to come in touch with the insect body. It is also noteworthy that stained grains were localised on the stigmas in groups.

Another survey (Table 4) demonstrated that of all pollen-bearing stigmas only 24% had caught a single dyed grain, whereas in 76% of the cases two or more grains were present. Since the gynoecium contains 2(-3) ovules the theoretical chance of efficacious fertilization is rather great.

Table 4. Distribution of stained pollen grains over the stigmas

36 stigmas (24.3%) with 1 grain
24 stigmas (16.2%) with 2 grains
24 stigmas (16.2%) with 3 grains
64 stigmas (43.3%) with 4 or more grains

The experiments described in the previous chapter render it highly probable that pollen treated with stains has practically the same properties as untreated pollen at least as far as its power of adhesion to the insect body and of its successful translocation is concerned. It is, in our opinion, not necessary to put its equivalence with regard to adhesion to the stigmatic surface to the test: the sticky stigmas readily retain all sorts of particles (some of which are larger than the pollen grains) and their capacity to capture pollen grains by their adhesive properties certainly exceeds the affinity of the pollen grains to the surface of the insect body. Various considerations render the same chances of being transferred by biotic agencies. Considering that untreated pollen seems to adhere slightly better to the insect body, the transfer of untreated pollen by flies can be accepted beyond reasonable doubt, even if the transfer of untreated grains from a donor spike to a receptor spike could not be directly demonstrated: untreated pollen found on receptor spikes may have been deposited by a fly coming from a donor spike but just as well from various other sources outside the experimental area.

One must bear in mind that the duration of the experiments was usually shorter than the time-span of the daily visiting period (although it always included the phase of optimum activity of the flies), so that an — albeit relatively small—part of the potential pollen transfer was not recorded. This only means that the rate of biotic pollen transfer is undoubtedly slightly higher than it appeared to be under our experimental conditions. The fact that the flies often move about on the same spike (and preen themselves) favours the incidence of geitonogamy, and so does their habit of frequently moving towards inflorescences in the immediate neighbourhood (which may well belong to the same individual of *Plantago* *lanceolata*), but our experiments have shown that effective cross-pollinations are by no means rare.

As far as the relative importance of efficiency of the biotic and the anemophilous form of pollen transfer is concerned, the present experiments do not permit a clear verdict. A specially adapted method (by means of fertilisation experiments?) would have to be worked out for the purpose.

An ecologically interesting and relevant circumstance is that the syrphid flies of the *Platycheirus-Melanostoma* group as a rule visit the *Plantago* inflorescences during the first hours of the morning (see the first chapter). At that time of the day the wind is often hardly noticeable or at least very weak, so that presumably the relative share of the biotic pollen transfer in respect of that by air currents will be greater than later during the day. Local differences in the relative rates of the two types of pollination associated with different environmental conditions and habitat variation (such as landscape and vegetation type and microclimatological differences) cannot be precluded. The question in how far these variations may be correlated with, or reflected in, modifications of the inflorescence is of interest. Delpino (1870, cited and discussed in Müller, 1873) thought he could recognise forms with differences in structural adaptation, which was accepted with some diffidence by the latter author.

One should never loose sight of the fact that a biotic transfer of pollen of *Plantago lanceolata* as described in the present paper can only take place where this plantain and the potential pollinators occur sympatrically, which need not always be the case. Field observations and experiments in other areas are indicated, and it will be necessary to extend the investigation by comparing the anthecological syndromes of other species of *Plantago* with the situation in *P. lanceolata.*

The technique for the dying of pollen for purposes of specific identification after a possible transfer can probably be improved and extended to be used effectively for the study of other cases in which different taxa and plants and other (potential) pollen vectors are involved. Coldwell (1951) has developed a useful method to study the dispersal pattern of air-borne pollen (of different species of conifers) by using pollen labelled with radioactive substances (isotopes). This technique is rather laborious, however, and other workers who have attempted to apply this method have not been successful (B. J. D. Meeuse, priv. comm.). The use of artificially stained pollen permits a more or less small-scale approach to various anthecological problems, more particularly in border-line cases between entomophily and anemophily.

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